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# In Vitro Evaluation of Anti bacterial Activity of Ethanolic root extract of *Glycyrrhiza glabra* on Oral microbes

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**Abstract**

The aim of the present study was to evaluate the anti bacterial activity of *Glycyrrhiza glabra* on selected oral microbes. Antibacterial activity of ethanolic root extract of Glycyrrhiza glabra was screened against *Streptococcus mutans, streptococcus sanguis, Streptococcus salivarius, Streptococcus mitis, and Lactobacillus acidophilus u*sing disc diffusion technique. The Minimum inhibitory concentration [MIC] and Minimum bactericidal concentration [MBC] of the extracts were also determined. The results of this study showed that the extracts at different concentrations exhibited anti bacterial activity against the bacterial species tested.

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***Key words:***
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## INTRODUCTION

Herbal medicines have been used for many years. There are innumerable types of indigenous plants that have been used by people for centuries in the treatment of many ailments. The history of such usage is long and well documented.[1] Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substance from other sources including plants.[2] Herbs with medicinal properties are useful and effective source of treatment for various diseases. Currently many studies are being conducted to know these herbs in depth. Screenings of medicinal plants

for antimicrobial activities are important for finding potential new compounds for therapeutic use. There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases.[3] The present study was to evaluate the antibacterial activity of ethanolic root extract of *Glycyrrhiza glabra* on selected oral microorganisms.

*Glycyrrhiza glabra*, also known as liquorice and sweet wood, is native to the Mediterranean and certain areas of Asia. It is a perennial herb which possesses sweet taste. The main taproot, which is harvested for medicinal use, is soft, fibrous, and has a bright yellow interior.[4] It was one of the most widely known medicines in ancient history, and records of its use include Assyrian tablets of around 2000 BC and cortisone has been found useful for arthritis and allergies. In addition licorice has been used for mild Addison's disease and other adrenal insufficiencies, such as hypoglycemia.[5] Licorice also acts like the hormone, ACTH, causing sodium retention, potassium depletion, and water retention. The herb contains glycyrrhizin, glycyrrhetinic acid, flavonoids, asparagine, iso-flavonoids, and chalcones. The glycoside, glycyrrhizin has a similar structure and activity as the adrenal steroids.[6,7] It also possess good anti bacterial,[8] anti fungal,[9] anti oxidant,[10] antitussive,[11] hepatoprotective[12] and anti inflammatory activity.[13] Historically, the dried rhizome and root of this plant were employed medicinally as an expectorant and carminative. It is used for treating upper respiratory ailments including coughs, hoarseness, sore throat and bronchitis.

## MATERIALS AND METHODS

### Plant material

The ethanolic root extract of *Glycyrrhiza glabra* was obtained from Green Chem Herbal Extract & Formulations. Bangalore.

### Test microorganisms

Bacterial strains used were *Streptococcus mutans, Streptococcus sanguis, Streptococcus mitis Streptococcus salivarius*, *Lactobacillus acidophilus*. The organisms were isolated using selective media Mutans -Sanguis agar [ Hi media M977], Mitis-Salivarius Agar [Hi media M257] and Lactobacillus MRS agar [Hi media M641] and maintained in nutrient agar slope at 4°C in department of Microbiology, Saveetha Dental College.

### Methodology

The extracts were prepared in the following concentrations in sterile water. 2.5mg/ml, 5mg/ml and 10mg/ml. 50µl of extract of different concentrations were loaded on sterile filter paper discs measuring 6mm in diameter, so that the concentration of the extract on each disc was 125µg, 250µg and 500 µg respectively. The discs were dried and kept aseptically

### Screening of antibacterial activity [Disc diffusion technique]

Broth culture of the bacterial strains compared to Mac Farland's standard [14,15] 0.5 were prepared. Lawn culture of the test organisms were made on the Muller Hinton agar [MHA-Hi media M1084] plates using sterile cotton swab and the plates were dried for 15 minutes. Filter paper discs loaded with different concentrations of the extract were placed on the respective plates. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimeters. Standard antibiotic discs of amoxicillin (30mcg/disc) and PenicillinG (30mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

### Determination of minimum inhibitory concentration

Macro broth dilution or tube dilution method was done to determine the Minimum inhibitory

concentration (MIC) of the extracts[16,17]. A series of two fold dilution of each extract ranging from 8mg/ml to 0.125mg/ml was made in Muller Hinton broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1998). 100µl of standard inoculum of the bacterial strains matched to 0.5 Mc Farland's standard were seeded into each dilution. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing the growth medium and the inoculum) .The tubes were incubated at 37°C for 24 hours and checked for turbidity. MIC was determined as the highest dilution (that is, lowest concentration) of the extract that showed no visible growth.

**Minimum Bactericidal Concentration (MBC)**

The MBCs were determined by selecting tubes that showed no growth during MIC determination; a loop full from each tube was sub cultured onto Muller Hinton agar plates and incubated for further 24 hours at 37°C. The least concentration, at which no growth was observed, was noted as the MBC

**RESULT AND DISCUSSION**

The antibacterial activity of the extracts at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the table 1. The minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] were also determined for the extracts and the results are given in table 2.

The ethanolic extract was more effective against *Streptococcus mutans* with a zone of inhibition of 22mm diameter (at conc500 µg.) and was less effective against *Streptococcus salivarius* with zone of inhibition of 16 mm (at conc.500 µg.) and among the other bacterial species studied *Streptococcus mitis and Lactobacillus acidophilus* showed a zone of inhibition of 20mm diameter (at conc. 500 µg.) both and *Streptococcus sanguis* showed inhibition zone of 19 mm diameter (at conc.500 µg.).

The MIC and MBC values of ethanolic extract was found to be low compared to aqueous extract. The ethanolic extract was found to have Low MIC and MBC values of 2mg/ml & 2mg/ml for both *Streptococcus mutans* and *Streptococcus sanguis*. With *Streptococcus salivarius,* ethanolic extract showed a higher MIC and MBC value of 4mg/ml & 8mg/ml and for *Streptococcus mitis* and *Lactobacillus acidophilus* it was 4mg/ml and4mg/ml. The lower MIC and MBC value is an indication of high effectiveness of the extract whereas higher MIC and MBC indicates the less effectiveness of the extract.

**Table 1:** Anti bacterial activity of ethanolic root extract of *Glycyrrhiza glabra*

| Extract | Conc [µg] | Zone of inhibition [in mm diameter] | | | | |
|---|---|---|---|---|---|---|
| | | B1 | B2 | B3 | B4 | B5 |
| | 125 | 15 | 12 | 10 | 14 | 13 |
| | 250 | 19 | 15 | 13 | 17 | 16 |
| | 500 | 22 | 19 | 16 | 20 | 20 |
| Penicillin G | 30mcg/disc | 24 | 21 | 22 | 26 | 21 |
| Amoxycillin | 30mcg/disc | 25 | 23 | 20 | 24 | 22 |

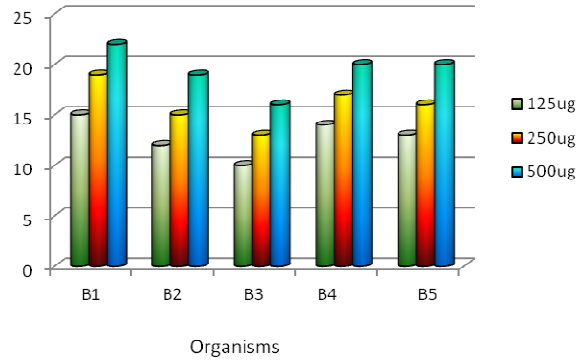
B1- *Streptococcus mutans*, B2- *Streptococcus sanguis*, B3- *Streptococcus salivarius,*
B4- *Streptococcus mitis*, B5- *Lactobacilus acidophilus*

**Table 2:** MIC and MBC of ethanolic root extract of *Glycyrrhiza glabra*

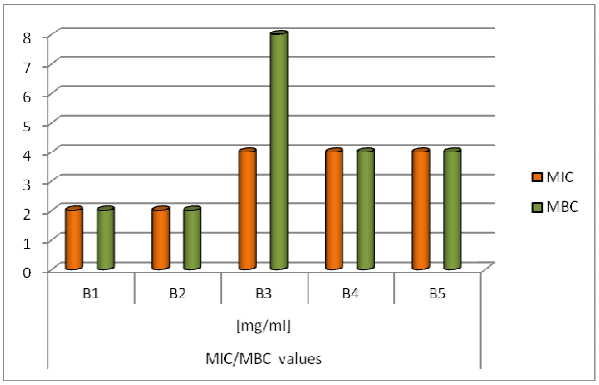
| Extract | | MIC/MBC values [mg/ml] | | | | |
|---|---|---|---|---|---|---|
| | | B1 | B2 | B3 | B4 | B5 |
| Ethanolic | MIC | 2 | 2 | 4 | 4 | 4 |
| | MBC | 2 | 2 | 8 | 4 | 4 |

B1- *Streptococcus mutans*, B2- *Streptococcus sanguis*, B3- *Streptococcus salivarius,*
B4- *Streptococcus mitis*, B5- *Lactobacilus acidophilus*

**Graph 1:** Anti bacterial activity of ethanolic root extract of *Glycyrrhiza glabra*




**Graph 2:** MIC and MBC of ethanolic root extract of *Glycyrrhiza glabra*




Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. The association between oral diseases and the oral microbiota is well established. Dental caries is a microbial disease that result in the destruction of mineralized tissue of the teeth. Streptococcus mutans is the potent initiator and leading cause of dental caries world wide .[18] It is considered to be the most cariogenic of all of the oral Streptococci. Another organism that is important in the development of caries is Lactobacillus acidophilus. This bacteria is not important in the initiation of caries but in the continuation. The present study was to evaluate the antibacterial activity of ethanolic root extract of *Glycyrrhiza glabra* against caries causing organisms. The results obtained from our study shows that ethnolic extract has got a very good antibacterial activity against the selected oral pathogens.

## CONCLUSION

The present results therefore offer a scientific basis for traditional use of root extract of *Glycyrrhiza glabra on* oral pathogens. The anti-bacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration. The use of herbs in dentistry should be based on evidence of effectiveness and safety. Although there may be benefits to using herbal medicines in the practice of dentistry, we really do not know much about them. With additional research, there definitely will be a niche for herbal treatments in dentistry

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